IMMUNOPATHOLOGY AND THERAPEUTIC MANIPULATION OF THE IMMUNE SYSTEM

Srinivas Kaveri
INSERM UMRS 872 Equipe 16, Centre de Recherche des Cordeliers
15, Rue de l'Ecole de Medecine, 75006 PARIS, France

Autoimmune diseases emerge as a result of a rupture in the immune tolerance mechanisms. Although most autoimmune conditions are associated with the presence of autoantibodies, autoaggressive T helper (Th) cells playa critical role in the immunopathogenesis of several autoimmune diseases such as rheumatoid arthritis (RA), type 1 diabetes and multiple sclerosis (MS). The effector functions of Th cells are regulated by their cytokine profile. The cytokines secreted by the antigen presenting cells (APC) determine the polarization of Th cells into Th1 or Th2 populations. Recent description of Th17 cells has lead to the reshuffling of the concept of the role of Th1 cells which were considered for a long time, as the major pathogenic Th cells in autoimmunity. Although these concepts have emerged basically from studies in experimental animal models, similar observations in other animals and man have led to the conception of novel therapeutic strategies, which will be highlighted in this presentation.

Introduction

Despite the stringent mechanisms that ensure the maintenance of immune tolerance, its breakdo-wn leads to a significant expansion of the self-reactive effector T cell population causing tissue-specific inflammation. Antigen presenting cells (APCs) play an important role in the initiation and progression of the pathological autoimmune response. APCs capture the antigens (Ag) and present them to the T cells as processed peptides in the context of human leukocyte antigen (HLA) or major histocompatibility complex (MHC). There are two classes of HLA/MHC class I presents peptides derived from the cytosol, while class II presents peptides derived from intracellular vesicles. HLA/MHC class I is found on nearly all cell types, and is responsible for presenting Ags derived from viruses, intracellular bacteria and tumors. HLA/MHC class II is only expressed on so-called professional APC, including dendritic cells (DC).

The role of APCs during an immune reaction, is orchestrated through three types of signals necess -ary for the activation of antigen-specific T cells. The first signal involves the presentation of antigen on the surface of an MHC class II molecule, which facilitates T cell recognition of the cognate antigen though the T cell receptor (TCR). A second signal is provided through the interaction of costimulatory and adhesion molecules present on the APC, such as CD80 and CD86, with CD28 expressed on the surface of T cells, and this cross-talk is crucial for activation and expansion of antigen-specific T cells. The third signal involves the secretion of cytokines by APCs, which directs the differentiation of activated antigen-specific lymphocytes into an effector T cell subtype. Current understanding of the T helper responses have allowed their classification into three T cell subsets: Th1, Th2 and Th17. Th17 cell subset described in detail below, has recently emerged as a third T cell subset that seems to play an important role in protection against certain extracellular pathogens (Bettelli *et al*., 2006; Mangan *et al*., 2006).

Th1-Th2 polarization

According to the initial classification proposed by Robert Coffman and Tim Mosmann in the late eighties, helper T cells were divided into two groups, Th1 and Th2, each with opposing activities, although currently this classification is considered to be an oversimplification of the system, and is subject to intense debate (Coffman, 2006). During their differentiation program, Th1 and Th2 cells lose the lymph node homing receptors and acquire the capacity to migrate to inflamed non-lymphoid tissues to execute their effector functions.

For a long time, the concept of the pathogenesis of autoimmune diseases and allergic diseases relied strongly on the Th1/Th2 subset model, according to which, Th1 cells are major pathogenic T cell subset in the context of tissue-specific T cell-mediated autoimmune diseases, while Th2 cells are responseb -le for the initiation of allergic reactions and asthma. IFN-g, one of the prototype cytokines of Th1 subset inhibits the differentiation and effector functions of Th2 cells, and can lead to a dominant Thl response. The APC-derived

cytokine IL-12 strongly drives the differentiation ofThl cells in vitro and in vivo, partly through its potent induction of IFN-g production. ,.

As a corollary, Th2 cells would down-regulate harmful Th1 cells and exert beneficial effects during an ongoing tissue-specific autoimmune inflammation. The counter regulatory role of Th1 and Th2 cells in autoimmune pathologies has been elucidated mainly through the experimental autoimmune encephalitis (EAE), an animal model for MS and collagen-induced arthritis, a model for RA. Thus, the Th 1- Th2 dogma of the early 1990s enforced the idea that Th 1 cytokines are deleterious in autoimmune disea -ses, such as multiple sclerosis and Type 1 diabetes, whereas Th2 cytokines are beneficial. A tilt in the ratio of the Th 1 ITh2 cytokines toward a more dominant Th2 environment that included significant upregulation of the Th2 cytokines IL-4, IL-5, and IL-10 was shown to be associated with protection from autoimmune inflammation (Starn *et al*., 1993; Adorini *et al*., 1996; Pashov *et al*., 1997). Accordingly several groups have explored the therapeutic potential of manipulating the cytokine balance.

Th17 cells and their role in autoimmunty

IL-17 is a proinflammatory cytokine that belongs to a family of six members: IL-17 (also
represented as IL-17 A), IL-17S, IL-17C, IL-17D, IL-17E and IL-17F (HarFington *et al*., 2005; Harrington *et al*., 2006). Initially identified in early nineties as a product of activated CD4+ T cells, recently IL-17 was recognized as a signature molecule of a subset of helper T cells, now referred to as Th17 cells (Iwakura and Ishigame, 2006). The expression of IL- 17 has been associated with several human autoimmune diseases, such as RA (Chabaud *et al*., 1999) and MS (Lock *et al*., 2002), and disruption of the encoding gene in the corresponding animal models has provided a varying degree of protection. In view of the importance of 11-17 in the pathogenesis of certain autoimmune and inflammatory diseases, mechanisms underlying the regulation of differentiation of this lineage as well as its precise role in the pathogenesis of autoimmune diseases, have been intensively investigated in various mouse models. Similar to Th 1 and Th2 T cell subsets, Th 17 differentiation is also controlled by products of DC and other APCs. Cytokines such as IL-6 and TGFa are important initiators of the differentiation of naive CD4+ T cells into Th 17 cells. Further, a delicate balance of cytokines controls the differentiation of naive CD4+ T cells into either polarized Th 17 cells or autoimmunity-suppressing regulatory T cells (Treg). While TGF-a stimulates polarization of CD4+ T cells into Tregs in the absence of IL-6 in vitro, the additional presence of IL-6 shifts the balance toward the pro-inflammatory Th 17 phenotype, a process that is amplified by IL-1 a and is negatively regulated by Th1 and Th2 cytokines (Stockinger and Veldhoen, 2007). Both in vitro and in vivo differentiation of Th 17 cells require induction of the transcription factor retinoic acid-related orphan receptor at (RORat), which is characteristic of the Th17 subset (Ivanov *et al*., 2006).

The importance of TGF-β plus IL-6 in differentiation of Th17 cells is further confirmed in vivo in that the immunization of TGF- β transgenic mice with MOG in CFA results in an enhanced Th 17 response and exacerbated EAE (Bettelli *et al*., 2006) while mice expressing a dominant negative form of the TGF- β receptor were deficient in Th17 cells and resistant to the development of EAE (Veldhoen *et al*., 2006). On the basis of these findings, it is proposed that there is a reciprocal relationship between Tregs and Th 17 cells in which IL-6 plays a pivotal role in dictating whether the immune response is dominated by pathogenic Th17 cells or protective Treg cells (Bettelli *et al*., 2006).

CD4+CD25+Foxp3+ regulatory T lymphocytes

Despite the existence of several stringent central tolerance mechanisms that ensure the elimination of T cell clones with high affinity for self peptide-MHC products, the mature T cell repertoire is not comple -tely free of self-reactivity. Peripheral processes such as activation-induced cell death and anergy may also fail to constrain the expansion of these T cells thereby leading to the initiation of pathogenic autoimmune responses. Efforts to identify additional peripheral mechanisms that may exert inhibitory effect on the proliferation of potentially pathogenic effector cells have resulted in the identification of a variety of supper -ssive or regulatory cells. Among the CD4+ T cell population, three prominent subtypes of suppressive T cells have been identified: T regulatory 1 cells (Tr1), which exert suppressive function in an IL-10-depende

-nt fashion, TGF- b-producing Th3 cells and naturally occurring CD4+CD25+ regulatory T cells (Tregs) (Bluestone and Tang, 2005). Among these populations, CD4+CD25+ Tregs have been extensively studied, and there is ample evidence that these cells contribute to both the maintenance of self-tolerance and to prevention of excessive responses against infection (Sakaguchi, 2005). Neonatal thymectomy or depletion of Treg from healthy animals leads to the manifestation of organ-specific autoimmune diseases in experime -ntal models, viz., inflammatory bowel disease, gastritis, type 1 diabetes, EAE or myocarditis. In these models, inflammation can be prevented by reconstitution of the animals using Tregs from wild type animals.

The best indication that either qualitative or quantitative defect in the pool of Tregs leads to an autoimmune condition comes from the observations of IPEX (immunodysregulation, polyendocrinopathy and enteropathy, X-linked syndrome). IPEX is a rare form of autoimmunity that occurs due to mutations in the transcription factor forkhead box P3 (FoxP3). Decrease in the numbers or impairment in the functional activity of Tregs, in peripheral blood or target organs, have also been associated with several human chroni -c inflammatory and autoimmune diseases including multiple sclerosis (Viglietta *et al*., 2004). RA (Cao *et al*., 2004; Ehrenstein *et al*., 2004), type 1 diabetes (Kukreja *et al*., 2002), inflammatory bowel disease (Makita *et al*., 2004), systemic lupus erythematosus (Crispin *et al*., 2003), polyglandular syndrome type II (Kriegel *et al*., 2004), myasthenia gravis (Balandina *et al*., 2005) and dermatomyositis (Ling *et al*., 2004).

In conclusion, in recent years, a significant progress has been achieved in our understanding of the dynamic communication within the cellular compartment, between newly identified effectors and several types of regulatory cells. Data regarding the underlying mechanisms are emerging at great speed. Technical advancement has allowed the development of a wide array of tools to expand therapeutically meaningful Tregs or to identify molecules that can be exploited towards enforcing suppressive functions of regulatory T cells to curb the pathogenic autoimmune inflammation. The real challenge now would be to determine whether Tregs can provide the focus for more effective therapies for autoimmune diseases. Approaches aimed at modulating the immune system through manipulating various molecular partners in order to attain a therapeutic benefit are emerging at a rapid pace and offer novel means of combating the painful and fatal diseases (Figure 1).

Reference

Bettelli , E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L. and Kuchroo, V. K.:2006, Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, **441**: 235-238.

Mangan, P. R, Harrington, L. E., O'Quinn, D. B., Helms, W. 5., Bullard, D. C., Elson, C. O., Hatton, R. D., Wahl, S. M., Schoeb, T. R and Weaver, C. T. :2006, Transforming growth factor-beta induces development of the T (H)17 lineage.*Nature*, **441**:231-234.

Coffman, R. L.:2006, Origins of the T(H)1-T(H)2 model: a personal perspective. *Nat.Immunol*, **7**: 539-541.

Starn, W. B., Van Oosterhout, A. J. and Nijkamp, F. P.:1993, Pharmacologic modulation of Th1- and Th2-associated Iymphokine production. *Life Sci,* **53**: 1921-1934.

Adorini, L., Guery, J. C. and Trembleau, S.:1996, Manipulation of the Th1/Th2 cell balance:an approach to treat human autoimmune diseases? *Autoimmunity*. **23**: 53-68.

Pashov, A., Bellon, B., Kaveri, S. V. and Kazatchkine, M. D.:1997, A shift in encephalitogenic T cell cytokine pattern is associated with suppression of EAE by intravenous immunoglobu -lins (IVlg). *Mulf. Seier*.**3**: 153-156.

HarFington, L. E., Hatton, R D., Mangan, P. R, Turner, H., Murphy, T. L., Murphy, K. M. and Weaver, C. T.:2005, Interleukin 17 -producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol*. **6**: 1123-1132.

Harrington, L. E., Mangan, P. R. and Weaver, C. T.:2006, Expanding the effector C D4 T-cell repertoire: the Th17 lineage Curr Opin Immunol. **18**: 349-356.

Iwakura, Y. and Ishigame, H.:2006, The IL-23/IL-17 axis in inflammation. *J Clin Invest*.**116**: 1218-1222.

Chabaud, M., Durand, J. M., Buchs, N.,Fossiez

F., Page, G., Frappart, L. and Miossec P.:1999, Human interleukin-17: AT cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arlhritis Rheum*.**42**:963-970.

Lock, C., Hermans, G., Pedotti, R, Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober,S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S. J., Oksenberg, J. R, Raine, C. S.:2002, Heller, R and Steinman, L., Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomy -elitis. *Nat Med*. **8**: 500-508.

Stockinger, B. and Veldhoen, M.:2007, Differentiation and function of Th17 T cells. Curr Opin *Immunol*. **19**: 281-286.

Ivanov, II, McKenzie, B.S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lataille, J. J., Cua, D. J. and Littman, D. R.: 2006, The orphan nuclear receptor RORgammat directs the differentiation program of pro inflammatory IL-17+ Thelper cells. *Ce1l.* **126**: 1121-1133.

Veldhoen, M., Hocking, R J., Flavell, R A. and Stockinger, B.:2006, Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol*. **7**: 1151-1156.

Bluestone, J. A. and Tang, Q.:2005, How do CD4+CD25+ regulatory T cells control autoimmunity? *Curr Opin Immunol*. **17**: 638-642.

Sakaguchi,S.:2005, Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol*. **6**: 345-352.

Viglietta, V., Baecher-Allan, C., Weiner, H. L. and Hatler, D. A.:2004, Loss of functio -nnal suppression by CD4+CD25+Regu -latory T cells in patients with multiple sclerosis. *J Exp Med*.**199**: 971-979.

Cao, D., van Vollenhoven, R, Klareskog, L., Trollmo, C. and Malmstrom, V.:2004, CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease. *Arthritis Res Ther*. **6**: R335-346.

Ehrenstein, M. R, Evans, J. G., Singh, A., Moore,S., Warnes, G., Isenberg, D. A. and Mauri, C.:2004, Compromised function of regulatory T cells in rheumatoi -d arthritis and reversal by anti-TNF alpha therapy.*J Exp Med*.**200**:277-285.

Kukreja, A., Cost, G., Marker, J., Zhang, C., Sun, Z., Lin-Su, K., Ten,S., Sanz, M., Exley, M., Wilson, B., Porcelli, S. and Maclaren, N.:2002, Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest*. **109**: 131-140.

Makita, S., Kanai, T., Oshima,S., Uraushihara, K., Totsuka, T., Sawada, T., Nakamura, T., Koganei, K. Fukushima, T. and Watanabe, M.:2004, CD4+CD25bright T cells in human intestinal lamina propria as regulatory cells. *J Immunol*. **173**: 3119-3130.

Crispin, J. C., Martinez, A. and Alcocer-Varela, J.:2003, Quantification of regulatory T cells in patients with systemic lupus eryt -hemaatosus *J Autoimmun*.**21**:273-276.

Kriegel, M. A., Lohmann, T., Gabler, C., Blank, N., Kalden, J. R and Lorenz, H. M.:2004, Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. *J Exp Med*. **199**: 1285-1291.

Balandina, A., Lecart, 5., Dartevelle, P., Saoudi, A. and Berrih-Aknin, S.:2005, Functional defect of regulatory CD4(+)CD25+ T cells lnthe thymus of patients with autoimmune myasthenia gravis. *Blood*. **105**: 735-741.

Ling, E. M., Smith, T., Nguyen, X. D., Pridgeon, C., Dallman, M., Arbery, J., Carr, V. A. and Robinson, D. S.:2004, Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet.* **363**: 608-615.

♀♀♀♀♀♀♀